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STUDIES OF PROBLEMS RELATED TO THE PRODUCTION OF 2,3-BUTYLENE GLYCOL BY AEROBACTER FERMENTATION.

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University of Alberta

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STUDIES OF PROBLEMS RELATED TO THE PRODUCTION OF 2,3-BUTYLENE GLYCOL BY AEROBACTER FERMENTATION

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STUDIES OF PROBLEMS RELATED TO THE PRODUCTION OF 2,3-BUTYLENE GLYCOL BY AEROBACTER FERMENTATION

W. C. HINMAN

INTRODUCTION

The critical rubber shortage which developed among the Allied Nations during the early years of the present world conflict when Japan seized the major areas of natural rubber production has resulted in the extensive development of the synthetic rubber industry. Several methods have been developed for the production of synthetic rubber, most of them using by-products from the petroleum industry. In view of the possible exhaustion of this source and the increasing demand for rubber products together with the necessity of developing new uses for surplus agricultural products, interest was directed toward methods involving the use of grain and other agricultural products as sources of raw material.

Synthetic rubber is made by polmerizing a mixture of 75 parts of butadiene with 25 parts of styrene. Butadiene the basic material is derived from three main sources:

1. Petroleum.
2. Ethyl alcohol.
3. 2,3-butylene glycol.

SYNTHETIC RUBBER FROM 2,3-BUTYLENE GLYCOL

J. C. HILLYER

INTRODUCTION

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Synthetic rubber is made by copolymerizing a mixture of 75 parts of butadiene with 25 parts of styrene. Butadiene the basic material is derived from three main sources:

1. Petroleum.
2. Ethyl alcohol.
3. 2,3-butylene glycol.

Alcohol and 2,3-butylene glycol (referred to henceforth as glycol) can be produced from grain by fermentation.

Efforts were directed toward the development of a process for the production of glycol from grain commercially by bacterial fermentation. Although the method was not developed in time to become a factor in sythetic rubber production it still has commercial possibilities. Butadiene of very high purity, far exceeding that derived from alcohol, can be produced by this method in a simple and direct manner. Also after the war this bacterial fermentation process may develop into a commercial source of glycol since glycol has many potential industrial uses which, though not yet intensively explored, appear very promising and 2,3-butylene glycol may replace ethylene and other glycols in many applications.

This investigation was conducted in both Canada and the United States in close cooperation with one another and was centralized at the Northern Regional Research Laboratory at Peoria. Under the direction of the National Research Council at Ottawa the Soils and the Plant Science departments of the University of Alberta undertock a joint laboratory study of the fermentation process for producing glycol.

Two types of fermentation were studied under this investigation -- one involving Aerobacillus polymyxa which ferments starch directly and produces the levo form of glycol, and the other Aerobacter aerogenes which ferments sugar, the

starch being converted to sugar by a saccharifying agent. The latter produces the meso form of glycol. Formerly Aerobacillus fermentation was studied by the division of Plant Pathology, Department of Plant Science and the Aerobacter fermentation by the division of Soil Microbiology of the Soils Department, the analytical work being done by the Biochemical division of the Department of Plant Science. Later when the main laboratory phase of the investigation neared completion anaylsis of the fermentation liquors was done by the respective groups who carried out the fermentations.

Different phases of the project have been reported on by A.L. Shewfelt, A.W. Jackson, W.G. Corns and A.C. Blackwood in their respective theses so that all three sections of the project were completely covered. This thesis represents a continuation of the section reported on by Blackwood -- namely Aerobacter fermentation.

REVIEW OF LITERATURE

The production of 2,3-butylene glycol by fermentation was first discovered in England in 1904. Since then it has been found that at least three different species of bacteria produce this compound during fermentation, among them the Aerobacter genus. According to Bergey's Manual of Determinative Bacteriology (2) Aerobacter aerogenes the type species is listed as a short rod, 0.5 to 0.8 by 1.0 to 2.0 microns, occurring singly, non-motile, non-spore forming, frequently capsulated, gram negative and facultative aerobic.

Most sugars including glucose, maltose, sucrose, lactose, galactose, levulose and the pentoses are readily fermented by this organism. Starch and dextrins are fermented very slowly if at all. The products of fermentation according to Hardin and Walpole (9) and also Reynolds and Workmann (17) are 2,3-butylene glycol and small amounts of alcohol, acetylmethyl carbinol, formic, lactic, acetic and succinic acids. Also carbon dioxide and hydrogen gases are produced in small amounts in the ratio of 2:1. The metabolic breakdown of glucose in the fermentation has been investigated and discussed by several investigators (1, 13).

Among the early investigators who attempted to perfect the fermentation were Verhave (23) who developed a process using Clostridium polymyxa or Aerobacter aerogenes, and Fulmer et al (8) using Aerobacter pictinovium. Kendall tested four organisms that gave similar results and he lists several factors

affecting the fermentation. He estimated that 50% of the sugar consumed is converted to glycol by this fermentation. Kluyver (13) at Delft University in Holland, patented in 1924 a method of producing 2,3-butylene glycol commercially by fermentation using a simple carbohydrate media.

The Northern Regional Research Laboratory of the United States Department of Agriculture, in cooperation with several industrial laboratories, was the first to begin a large scale intensive investigation to develop the commercial aspects of this fermentation. Using their strain which they isolated early in the project and designated as A. aerogenes #199 they were able to obtain glycol yields which approached the maximum amount obtainable. They developed an agar medium for storing the organism and standard methods of handling the culture (16). Other laboratories (3, 18, 22) isolated and tested many other strains of A. aerogenes but no isolate was found which was superior in fermenting ability to #199. Seagrams (4) in 1944 found that strain #766 isolated at the University of Wisconsin was more valuable than #199. This strain was used in their pilot plant tests and found very satisfactory.

It has been quite generally accepted that 5% inoculum be used in the fermentation although some laboratories (16) have suggested using up to 10%. Transferring directly from the slant to the inoculum media gave better yields than frequent transferring from liquid media (18), although the fre-

quently transferred gave a more vigorous growth. The University of Wisconsin (22) found that age and size of the inoculum made very little difference and they preferred using 5% of a 12 hour inoculum. Seagrams (11) obtained best results with a 20 hour inoculum. Heyden Chemical Corporation (10) suggest using an inoculum shaken 18 hours at 90 cycles per minute, at 32° C. The Schenely Research Institute (19) got consistently higher yields of glycol by carrying the cultures from an agar slant through four generations on synthetic medium and then one generation on cooker mash, the final culture being aerated to acclimatize the organism to this treatment. Seagrams (11) suggest that their rich synthetic inoculum is superior to N.R.R.L. corn steep liquor medium. University of Wisconsin also found Seagrams' inoculum to give higher yields of glycol.

A great deal of work has been done to develop an optimum fermentation medium. It was found (16) that small amounts of magnesium sulphate, about 0.025%, and of potassium acid phosphate, about 0.180%, were essential in glucose media. Seagrams used ammonia as a nitrogen source in their pilot plant investigations (4). When urea is used as the nitrogen source successful fermentations can be obtained without the use of calcium carbonate as a buffer, but when ammonium sulphate is used calcium carbonate is necessary since the medium becomes too acid for normal growth of the organism and both glucose consumption and glycol production are inhibited (16). It is quite generally

agreed that the calcium carbonate must be sterilized separately either dry or in a slurry since at the temperature of sterilization the calcium carbonate causes a partial transformation of the glucose, and the products formed are not fermentable by A. aerogenes. The N.R.R.L. found that salt additions were not necessary in malt hydrolyzed corn mash (16). This was confirmed by the Schenely Research Institute (18) if salts were used in the inoculum. Blackwood (3) reported a small increase in yield when salts were added to malt hydrolyzed wheat mash at the time of inoculation. The mash may be saccharified in either of two ways -- by the use of malt or by acid hydrolysis. Schenely Research Institute (18) found that malt hydrolyzed medium gave a faster fermentation with increased yields of glycol. This was also confirmed by Blackwood who found that calcium oxide or sodium carbonate were necessary in acid hydrolyzed media to maintain a proper pH. Malt hydrolyzed medium must be resterilized after hydrolysis because A. aerogenes cannot compete with the contamination introduced with the malt. The University of Wisconsin (22) reported that unsterilized malt sprouts added to synthetic medium resulted in a faster fermentation, but that heating destroyed this stimulating factor. Seagrams (11) found that malt sprouts added to grain mashes gave neither an increased yield nor a speed up in rate of fermentation; instead 2% unsterilized malt sprouts had a detrimental effect on the fermentation due to contamination introduced into the medium.

A fermentation temperature of 30°C has been suggested by most investigators as the optimum. The N.R.R.L. (16) found that a temperature of 37°C gives a more vigorous fermentation but results in reduced glycol yields. This is confirmed by Schenely Research Institute (19). Heyden Chemical Corporation (10) reports that within a range of 31 to 35°C, the temperature of incubation has no significant effect on the yield of glycol and carbinol, but that the rate of fermentation is fastest at the highest temperature.

Aeration is one of the most important single variable factors affecting the fermentation. Its exact role is not clearly understood although it is quite generally accepted that it exerts a physical rather than a chemical action. Although the organism is classified by some as an obligate aerobe no oxygen is needed to balance any equations representing the breakdown of sugar to glycol (13, 17). The National Research Council at Ottawa (15) reported that nitrogen may replace air and give a normal fermentation, but this has not been verified by other laboratories. In small batches mechanical agitation gave yields comparable to those obtained when the mash was aerated but this could not be reproduced in large batches (19). Stirring with a mixer has been reported to give good results in small batches (8, 17, 22). In larger samples agitation and aeration together gave a normal fermentation (16).

The rate of aeration is an important factor and has received much attention by numerous investigators. The conditions affecting the rate of aeration are: volume of medium, distance through which the air must travel, type of fermentor, stage of fermentation and desired time of fermentation. The Schenely Research Institute suggests a rate of 0.01 to 0.05 cu.ft. per minute per litre of mash. Heavy rates of aeration speed up the fermentation but cause oxidation of part of the glycol to acetylmethyl carbinol especially towards the end of the fermentation. They also suggest that the fermentation takes place in two stages -- the first one aerobic and the latter one anaerobic. This anaerobic stage reduces the yield of acetylmethyl carbinol and increases the glycol yield, and sugar continues to be consumed. Aeration should be discontinued when the residual sugar reaches 3.0%. The N.R.R.L. (16) suggests a rate of 20 cc per litre per minute in a rotary drum fermentor and 200 cc in a vat fermentor. The Schenely Research Institute (19) reports optimum yields using 510 cc per litre per minute and they got higher yields when the aeration was reduced to half after 36 hours. Seagrams (11) suggest an aeration rate of.02 cu.ft. (567 cc) per minute per litre to be the optimum. Aeration for the whole fermentation gave better results than when discontinued during the latter part of the fermentation. Aeration is more effective when the air is broken down into small bubbles (10,23) and various aerators have been suggested. Less air is required in large

scale operation than in laboratory size tests. Seagrams (4) got higher yields in their pilot plant when aeration with agitation was used than with aeration alone. Proper agitation reduced the air requirements from 6.6 cu.ft. to 3.0 cu.ft. per minute per 1000 gallons of mash.

Several investigators have studied the effect of metalic ions on the growth of the organism. Seagrams (11) found that copper was most toxic and was fatal in concentrations of 0.1 to 0.5 mg. per litre. Nickle, iron, zinc and chromium ions are inhibitory to a certain extent. Manganese, silver and mercury ions are stimulating in low concentration but inhibitory in high concentrations. The University of Wisconsin (22) reported that copper in concentrations up to 2.0 mg. per litre and iron up to 15.0 mg. per litre had little effect on the fermentation, except that low concentrations had a stimulating effect on the rate of fermentation.

Bactereophage was encountered during pilot plant studies both by Schenely Research Institute (18) and Seagrams (11). Investigation has shown the phage or toxin to be weak in nature since ferments set with large inocula have undergone successful fermentation.

There has not been much literature made available concerning pilot plant investigations of this project. However, the feasibility of large scale fermentation using Aerobacter was first demonstrated by the N.R.R.L. (16) in 1942 when they successfully fermented three 550 gallon lots of

glucose medium in a pilot plant. The glycol yield was 82% of theoretical. The Schenely Research Institute (18) obtained in their semi pilot plant a glycol yield of 14 to 15 lbs. glycol per bushel of corn. Seagrams conducted pilot plant investigations, first using makeshift equipment which proved unsatisfactory, and later using a specially constructed pilot plant. They developed and described a process which can be adapted to commercial operation for the production of 2,3-butylene glycol from wheat, and its conversion to butadiene (4). They obtained yields approaching the theoretical value of 50% of the sugar consumed. The following products were recovered -- 14.5 lbs. glycol, 0.83 lbs. ethyl alcohol and 1.05 lbs. acetymethyl carbinol per 56 lbs. bushel of wheat. Yields of 15.5 lbs. of glycol have been obtained per bushel of wheat in the pilot plant. These yields compare with 10 lbs. glycol and 6 lbs. alcohol per bushel of wheat using Aerobacillus fermentation.

OBJECTS OF THE INVESTIGATION

The objects of the investigation were as follows:

1. To maintain the original aim of the project, i.e., to develop a fermentation which would give a maximum yield of 2,3-butylene glycol in the shortest practical time.
2. To study some of the problems which had arisen out of this investigation such as -- aeration, prevention of foaming during aeration, storing and handling the organism Aerobacter aerogenes, and to make a comparison of different starchy or sugar materials as sources of raw material for the fermentation.

METHODS AND MATERIALS

Unless otherwise indicated 10% dextrose media were used for all fermentations, with added salts and urea as described by the N.R.R.L. (16) or else 15% malt hydrolyzed wheat mash. Fermentations were made in large test tubes containing 100 cc of medium. These were inoculated with 5% of 18 to 24 hour inoculum. The inoculum used was that developed by the Schenely Research Institute (18) and two transfers were made before seeding the fermentation medium. The tubes were aerated in series at the rate of 500 cc per minute per litre of medium, the same air leaving one tube and entering the next, thus insuring the same rate of aeration in each tube. This technique aided in minimizing differences in yield between replicates due to differences in rate of aeration. In nearly every instance the fermentations were allowed to run until the residual sugar had reached a level approaching 0.5% or less. This was to obviate the necessity of determining residual sugars on the fermented liquors, since time did not always permit sugar analysis on each sample. Since Aerobacter aerogenes fermentations are primarily concerned with the sole end product 2,3-butylene glycol, carbinol and alcohol determinations were omitted in the majority of tests.

All wheat mashes were malt hydrolyzed, as this eliminated the necessity of neutralizing the medium, in contrast to acid hydrolyzed mashes, and the fermenting cultures were much more easily buffered. Also more uniform results could be obtained using malt hydrolyzed mash than with acid hydrolyzed mashes.

The methods of analyses were those developed by the Plant Science department (5), with the exception of glycol determinations on fermented beet molasses. There was some substance or factor present in the beet molasses which interferred with the glycol determination, and it was found necessary to alter the procedure. Only one ml. of supernatent liquor was used for analysis instead of three as had been suggested, and the quantities of copper sulphate and calcium hydroxide were doubled. The amounts of acid and phosphate buffer were adjusted to produce a clear end point. The final result is multiplied by three. Also it was necessary to run a blank using unfermented beet molasses. Following this procedure good recovery of glycol was obtained from beet molasses solutions of known glycol concentration.

RESULTS

1. Aeration Studies

Aeration has proven to be one of the most important single variable factors affecting the fermentation; perhaps the most important. Its exact role is not entirely understood but its effect is very apparent and readily demonstrated. In previous work done at this laboratory the major portion of the variation between replicates in the same test was attributed to the ineffective control of the rate of aeration. Later during the investigation it was found that this variation between replicates could be very effectively but not wholly eliminated by aerating in series, the same air leaving one sample tube and entering the next. Consequently by using tightly fitting connections the rate of aeration in each tube was exactly the same and could be easily measured and controlled. This more reliable technique opened the way for further study on the effect of aeration. It was quite generally believed that aeration produced a physical rather than a chemical effect upon the fermentation. Therefore a series of experiments was planned using different gases as the aerating medium in order to investigate and substantiate these conclusions.

In these experiments N.R.R.L. dextrose medium (16) containing 10% dextrose was used and it was fermented in

TABLE 1 - Effect of aerating with different gases on the fermentation of dextrose medium.

Gas	No. of Samples	Time Hours	pH	Yield from 10 grams glucose Titratable Acidity$_x$	Glycol Grams	Alcohol Grams
Air	9	42-48	5.7	.42	3.95	.71
CO_2	9	60-70	5.5		3.12	.75
Air	9	40-48	5.8	.34	3.73	1.10
N_2	9	54-66	5.2	.50	2.63	1.28
Air	6	44-50	5.7	.50	3.43	1.16
H_2	6	54-66	5.1	.80	2.44	1.28
Air	3	44	5.6	.52	3.15	
None	3	110	5.4	.38	2.47	

x Expressed as grams lactic acid.

TABLE 1 - Effect of aerating with different gases on the fermentation of dextrose medium.

Gas	No. of Samples	Time Hours	pH	Titratable Acidity*	Yield from 10 grams glucose Glycol	Alcohol	
Air	9	42-48	4.7	.42	3.34		.71
CO_2	9	94-96	4.5		3.13		.79
Air	9	46-48	5.9	.34	3.73		1.30
N_2	9	94-96	5.7	.40	4.05		1.38
Air	6	44-50	5.7	.50	3.43		1.16
H_2	6	94-96	5.1	.80	3.49		1.28
Air	3	44	5.8	.32	3.15		
None	3	110	5.4	.37	3.47		

* Expressed as grams lactic acid.

large test tubes containing 100 cc of medium, and aerated at the rate of 500 cc of air per minute per litre of medium. The fermentation was allowed to run until the sugar had all been consumed (i.e. less than 1% remained in the fermentation liquor.) The liquors were then analysed for glycol and alcohol and the results are reported in Table 1. The gases used in these tests were air, nitrogen, hydrogen and carbon dioxide.

The results of this experiment show that the effect of aeration is primarily a physical one since in every instance the fermentation did go to completion (i.e. all the sugar was consumed) in a much shorter incubation period than when no aeration was employed. At the same time there is an indication that aeration has a chemical effect which although less marked seems to be important and should not be overlooked. Aeration with gases other than air retarded the rate of fermentation very noticeably, carbon dioxide retarding it more than hydrogen or nitrogen. Also it will be noted that the products of the fermentation were materially changed. The yield of glycol was much reduced while the titratable acidity (expressed as grams lactic acid) and alcohol were increased by the carbon dioxide, nitrogen and hydrogen. Carbinol was never found in the fermentation liquors when aerated with the pure gases whereas in the case of air there were

always traces of carbinol. It was noted that the carbinol increased if aeration was prolonged after the sugar was all consumed. This would seem to indicate that the glycol was oxidized to acetylmethyl carbinol. Stanier et al (20) have shown that Aerobacter aerogenes does oxidize glycol to carbinol, which they measured by the oxygen uptake. The fact that with aeration the fermentation will proceed to completion at an increased rate while at the same time the products of the fermentation are materially changed by the different pure gases indicates that the role of aeration is chemical as well as physical.

The process of supplying sufficient sterilized air to maintain the fermentation on a large scale would undoubtedly present a difficult problem to the commercial producer. Blackwood (3) in his investigation found that the same air could be recycled through ten tubes of fermenting medium without hampering the fermentation. The air entered the first tube and escaped from the last one. With the results of this experiment in mind a study was made to determine to what extent the same air could be recycled through the fermentation medium without impairing the glycol yields. The practical value of this experiment lies in the possibility of eliminating the necessity of sterilizing fresh air throughout the

fermentation period.

Since it was not possible in this laboratory practice to collect the exhaust gases and recycle them through the cultures, the fermentations were carried out using varying mixtures of carbon dioxide and air as the aerating medium. It was assumed that it was probably the accumulation of carbon dioxide in a non-aerated fermentation that accounted for at least part of the lack of efficiency in this type of fermentation. Although hydrogen is also produced during the fermentation it was not considered in this experiment since hydrogen gas is relatively inert. The following carbon dioxide and air mixtures were used:

100% carbon dioxide
75% carbon dioxide and 25% air
50% carbon dioxide and 50% air
25% carbon dioxide and 75% air
10% carbon dioxide and 90% air
0% carbon dioxide and 100% air.

Aeration with pure air was used to serve as a check. The aim was to determine the amount of carbon dioxide in the aerating medium which can be tolerated by the organism without affecting the fermentation detrimentally. The results of this experiment are recorded in Table 2 (A to F).

The results indicate that there was an increased glycol production as the ratio of air to carbon dioxide in the aerating medium increased, although the increase was not consistent particularly with the lower rate of aeration (250 cc per litre per minute). However air mixtures containing as much as 25% carbon dioxide had comparatively little deleterious effect on the ability of the organism to ferment sugar to glycol, and at least part of this lowered glycol production may be due to normal deterioration of the organism in its ability to ferment sugar to glycol (page 28) since the organism did undergo appreciable deterioration during the course of the experiments. The experiments were started with 100% carbon dioxide and carried on with progressively less carbon dioxide and more air. Pure air was also used at the beginning of the experiment. It is believed therefore that the organism Aerobacter aerogenes may tolerate an air mixture containing as high as 25% carbon dioxide without seriously affecting its fermenting efficiency. Providing a sufficient volume of air were used it seems quite possible that the same air could be used throughout the fermentation by recycling, and this would make it unnecessary to sterilize a very large volume of air.

This experiment brought out some other interest-

TABLE 2 - Effect of aeration with mixtures of carbon dioxide and air on the fermentation

Time	Rate of aeration cc per min. per litre		Yield from 10 grams glucose Glycol Grams	Alcohol Grams
A. - aeration mixture 100% CO_2 - 0% air.				
70 hours	500		3.28	.72
" "	"		2.92	.82
" "	"		3.15	.70
		Average	3.12	.75
" "	250		3.22	.72
" "	"		3.09	.72
" "	"		3.03	----
		Average	3.11	
B. - Aeration mixture 75% CO_2 - 25% air.				
67 hours	500		3.18	1.16
" "	"		3.36	1.06
" "	"		3.37	1.10
		Average	3.30	1.11
" "	"		3.74	1.01
" "	"		3.57	1.48
" "	"		3.47	1.65
		Average	3.58	1.38
C. - Aeration mixture 50% CO_2 - 50% air.				
66 hours	500		3.69	.95
" "	"		3.74	1.07
" "	"		3.68	1.03
		Average	3.70	1.02
" "	"		3.86	.86
" "	"		3.66	1.21
" "	"		3.12	1.16
		Average	3.55	1.08

TABLE 2 - Effect of aerating with electric arc carbon dioxide and air on the fermentation

Time	Rate of aeration cc per gr. per 11 hrs	Glycol	Alcohol	Organic
A. - Aerating mixture 10% CO_2 - 90 % air.				
70 hours	500	3.85		.95
" "	"	2.95		.85
" "	"	3.14		.70
	Average	3.31		.75
" "	250	3.05		.75
" "	"	3.09		.75
" "	"	2.8		
	Average	3.31		
B. - Aerating mixture 10% CO_2 - 90 % air.				
67 hours	500	3.15		1.14
" "	"	2.94		1.06
" "	"	2.99		1.10
	Average	3.30		1.11
" "	"	2.74		1.01
" "	"	3.47		1.28
" "	"	3.47		1.04
	Average	3.23		1.11
C. - Aerating mixture 10% CO_2 - 90 % air.				
66 hours	500	1.49		.95
" "	"	3.74		1.07
" "	"	3.02		1.03
	Average	3.70		1.02
" "	"	1.80		.84
" "	"	1.86		1.31
" "	"	1.31		1.10
	Average	3.33		1.08

TABLE 2 - Continued.

Time	Rate of aeration cc per min. per litre.		Yield from 10 grams glucose Glycol Grams	Alcohol Grams
D. - Aeration mixture 25% CO_2 - 75% air.				
54 hours	500		3.63	.53
" "	"		3.94	.66
" "	"		4.06	.51
		Average	3.88	.57
" "	250		3.44	1.30
" "	"		3.63	1.21
" "	"		3.86	.95
		Average	3.64	1.15
E. - Aeration mixture 10% CO_2 - 90% air.				
50 hours	500		3.99	.86
" "	"		3.83	.85
" "	"		3.81	
		Average	3.88	.86
" "	250		3.30	1.44
" "	"		3.35	1.32
" "	"		3.44	
		Average	3.36	1.38
F. - Aeration mixture 0% CO_2 - 100% air.				
48 hours	500		3.95	.53
" "	"		3.75	.95
" "	"		4.15	.66
		Average	3.95	.71
" "	250		3.90	.70
" "	"		3.87	.67
" "	"		4.00	.61
		Average	3.93	.66

ing facts. There is an indication that more glycol is produced by the higher rate of aeration (500 cc per litre per minute), although the differences may not be statistically significant. Also the lower rate of aeration (250 cc per litre per minute) had a tendency to increase the amount of alcohol produced; again the results are not entirely consistent and may not be significant.

2. Prevention of Foaming During Aeration.

Foaming of the medium during aeration created quite a problem, especially when using high rates of aeration averaging around 500 cc per minute per litre of medium. This tendency was greatest in malt hydrolysed products, both filtered and unfiltered (3). It was more noticeable when sodium carbonate was used as a buffer to neutralize the excess acidity. Several anti-foam agents, including amyl alcohol, commercial lard oil and oleic acid, had been used to conteract this tendency and although they were quite effective it was felt that they inhibited the fermentation to a greater or less extent.

It was believed that the foaming was caused by the proteins present in the hydrolyzed wheat mash and since acid hydrolyzed medium showed less tendency toward foaming than did the malt hydrolyzed medium it was surmised that the foaming was due to the unhydrolyzed proteins. The proteins would undergo a greater degree of hydrolysis in the acid medium than in the malted medium. With this in mind a small amount of proteolytic enzyme (trypsin) was added with the malt to a two litre batch of wheat mash during the malt saccharifying process. The resulting medium showed no tendency toward foaming even when subjected to extremely high rates of aeration. The

medium to which no enzyme had been added foamed even at moderate aeration rates. This experiment substantiated the theory that it was the unhydrolyzed proteins that caused the foaming.

Another object was to find an anti-foaming agent which did not have an inhibitory effect on the fermentation. Therefore, an experiment was carried out to compare the effect of hydrolysing the protein with that of adding anti-foaming agents. The anti-foaming agents used in this experiment were oleic acid and lubricating oil. For a check tubes were fermented without the addition of an anti-foaming agent. Five drops of the respective anti-foaming agent were added to the reaction tubes at the time of inoculation. The results of this experiment are reported in Table 3.

This experiment was repeated on different days and reasonably consistent results were obtained each time. The use of oleic acid gave glycol yields which although slightly lower were probably not significantly lower than those of the check, and sugar utilization was just as rapid. Lubricating oil and trypsin gave yields which were higher, though not consistently higher, than those of the check. The alcohol yield was slightly lower with both of these agents. At the rate of aeration used in this experiment (750 cc per litre per minute) the use

TABLE 3 - Effect of different anti-foam agents on the fermentation of malt hydrolyzed wheat medium.

Agent	Date		Yield from 15 grams wheat Glycol Grams	Alcohol Grams
None	June 14		3.21	
"	Aug. 16		3.48	.94
"	Aug. 30		2.40	
		Average	3.36	
Oleic Acid	June 14		3.16	
" "	Aug. 16		3.44	.96
" "	Aug. 30		3.34	
		Average	3.31	
Lubricating Oil	June 14		3.22	
" " "	Aug. 16		3.88	.84
" " "	Aug. 30		3.49	
		Average	3.53	
Trypsin	June 14			
"	Aug. 16		3.80	.78
"	Aug. 30		3.53	
		Average	3.66	

of oleic acid had no marked inhibitory effect on the fermentation. Lubricating oil, however, can be used to replace oleic acid as an anti-foaming agent and may prove of superior value in this respect because it not only gives more favorable yields but is also much cheaper to employ. Enzyme hydrolysis is quite effective and may have advantages in laboratory practice, yet its use commercially is questionable because of its high cost, and the large amounts which would be required in large scale commercial practices.

TABLE 4 - Effect of different anti-foam agents on the

Composition of malt monolayered tower medium.

Agent	Date	Trial	Control
None	June 14	3.42	
"	Aug. 24	3.40	.84
"	Aug. 30	3.40	
	Average	3.40	
Oleic Acid	June 14	3.10	
" "	Aug. 24	3.44	.46
" "	Aug. 30	3.34	
	Average	3.29	
Cottonseed Oil	June 14	3.40	
" " "	Aug. 24	3.68	.84
" " "	Aug. 30	3.60	
	Average	3.57	
Tryptin	June 14	3.40	
"	Aug. 24	3.60	.76
"	Aug. 30	3.72	
	Average	3.60	

of oleic acid had no marked inhibitory effect on the fermentation. Cottonseed oil, however, had to be used in place of oleic acid as an anti-foaming agent and was found to be very valuable in this respect because it not only gives more economic yields but is also much cheaper to obtain. Tryptone appears to be quite effective and may have commercial importance, yet its use economically is questionable because of its high cost, and the large amount which would be required in large scale commercial operations.

It was found by experiment that at least 0.01 grams of trypsin (not standardized) was required per litre of mash to effectively stop foaming in four hours when applied to an 18% wheat mash.

An experiment was conducted in an attempt to produce a malt which would sufficiently break down the protein during the malt hydrolyzing process to prevent foaming. The heat treatment in drying distiller's malt inactivates most of the proteolytic enzymes present in the malted barley (24). Therefore a malt was produced in the laboratory and dried at a comparatively low temperature (40°C). Tests were made using this malt and also distiller's malt as a check. The malt which had been dried at 40°C did not materially check the foaming even after mixing with the starchy material and standing as long as 36 hours in an oven at 55°C.

3. "Lyophilization" as a Method of Storing and Handling the Culture.

It became apparent that the Aerobacter aerogenes organism when stored on agar medium frequently undergoes a degenerative variation losing part of its ability to utilize and ferment glucose to glycol. This variation was suspected early in the investigation and was reported both by this laboratory and the National Research Council at Ottawa. The Schenely Research Institute (18) also reported a degenerative tendency of the organism which they believe was due to mutation. Variation of this type is characteristic of organisms which undergo rapid prolification. This degeneration occurred even when the organism was stored on slants in the refrigerator at 2°C without transferring. There is not only a decline in the activity of the organism but also a marked lowering of the glycol: alcohol production ratio, the ratio becoming lower as time elapses. Blackwood (3) has recorded a drop in the glycol: alcohol ratio of from 6.4:1.0 to 2.8:1.0.

To obviate this degenerative tendency and maintain the culture in good condition, i.e. produce glycol in yields approaching theoretical, a modified "lyophile" method of drying the culture was tried. This method was suggested by the National Research Council at Ottawa and described by Dr. G. A. Ledingham (14) and is a modifica-

tion of the process developed by Flosdorf and Mudd (7). It consists primarily of rapidly freezing the culture in small tubes and drying under vacuum from the frozen state. The tubes are sealed under vacuum and stored at room temperature until required. That the organism could be stored and maintained in this condition seemed quite likely. Elser et al (6) have shown that bacteria can be maintained in a viable condition in this state for an almost indefinite period. Moreover, they have preserved such sensitive forms as meningococci in a viable state for a period of 18 years, and in no instance did they find that strains deviated after storage even in minor particulars from their behavior before they were subjected to the drying process.

Several strains of the organism having different fermentative ability were "lyophilized" at this laboratory--some by A.C. Blackwood in January, 1944, and some by the author in May, 1944. These cultures retained their viability when tested three to four weeks after drying. When tested again after eight months they would not grow when transferred to nutrient broth. Legume nodule bacteria dried at the same time produced active cultures when transferred to a liquid medium. This was in agreement with results obtained at the National Research Council, Ottawa, where all dried cultures tested except Aerobacter aerogenes kept very well.

in this condition. This would indicate the lack of ability of Aerobacter aerogenes organism to remain alive over a long period in the dried state.

4. Urea as the Nitrogen Source.

There was some doubt as to how much urea was required for optimum fermentation. The N.R.R.L. (16) reported that 0.1 to 0.2% urea is sufficient in synthetic media. The Schenely Research Institute (18) suggested using 0.3% in dextrose medium and 0.2% in hydrolyzed mash medium. An experiment was conducted in which varying amounts of urea were added to the fermentation media. Tests were made on both dextrose medium and malt hydrolyzed wheat mash. The results appear in Tables 4 and 5.

On dextrose medium the greater the addition of urea the faster and more efficient was the fermentation (see Table 4). This may be due to two factors:

1. There was a larger supply of available nitrogen.

2. The greater the addition of urea the more nearly was the reaction of the medium maintained at a pH optimum for the growth of the organism.

In this experiment the maximum urea level was not reached. A similar experiment conducted by Blackwood showed that 0.5% of urea reduces the fermentation time to a minimum.

Table 5 shows that the optimum amount of urea in hydrolyzed wheat medium is 0.3%. Above this amount there was no increase in the rate of fermentation nor the glycol yield. Below 0.3% there was a definite drop in the rate of

4. Discussion of Results.

There was some doubt as to how much urea was required for maximum conversion. The B.A.E.L. (16) reported that 0.1 to 0.2% urea is sufficient in synthetic media. The Kennedy Research Laboratories (18) suggested using 0.5% in defibrate medium and 0.2% in corncobed wood medium. An experiment was conducted in which varying amounts of urea were added to the fermentation media. Tests were made on both defibrate medium and malt byproduced wheat straw. The results appear in Table 4 and 5.

In defibrate medium the presence of urea did not increase the fermentation rate. However, urea did increase the fermentation efficiency and more efficient use was made of the sugars (see Table 4). This may be due to two factors:

1. There was a larger supply of available nitrogen.

2. The presence of urea in the medium may have caused the formation of the reducing substance which is a co-enzyme for the growth of the organism.

In malt extract-bulk SO2 medium urea had little or no effect on the rate of fermentation. However, urea did increase the efficiency of the fermentation. The 0.5% urea level was found to be the most economical. Below 0.5% there was a definite drop in the rate of

TABLE 4 - Effect of various amounts of urea on the fermentation of dextrose medium.

UREA grams per 100 cc medium	Final pH	No. of Samples	Grams Glycol from 10 grams dextrose After 40 hours Residual Sugar	After 40 hours Glycol	After 48 hours Residual Sugar	After 48 hours Glycol
.1	4.7	3	3.36	3.10	2.41	3.47
.2	5.0	3	2.25	3.30	1.26	3.57
.3	5.5	3	.40	3.67	trace	3.72
.4	5.7	3	trace	3.90		3.93

TABLE 5 - Effect of various amounts of urea on the fermentation of malt hydrolyzed wheat medium.

UREA grams per 100 cc medium	Final pH	No. of Samples	Grams Glycol from 15 grams wheat after 42 hours	after 66 hours	after 80 hours
.1	5.4	3	1.38	1.95	2.29
.2	5.5	3	1.95	2.84	3.32
.3	5.6	3	2.16	3.25	3.86
.4	5.8	3	1.82	3.22	3.81

fermentation and yield of glycol. The effect of adding varying amounts of urea is apparent early in the fermentation and shows up throughout the whole fermentaion period.

It was noticed that the higher the initial urea level the higher was the final pH of the culture, so an experiment was carried out to determine the effect of omitting the calcium carbonate as a buffer agent when urea is added to the medium. The N.R.R.L. (16) obtained successful fermentations without the use of calcium carbonate as a buffer when urea was used as the nitrogen source. Blackwood found it necessary to use both calcium carbonate and sodium carbonate to maintain a proper pH when fermenting hydrolyzed wheat mash with urea as the nitrogen source. In this experiment a 15% malt hydrolyzed wheat mash was fermented and two levels of urea were used. The results are shown in Table 6.

When 0.3% urea is used there is no apparent detrimental effect from omitting the calcium carbonate buffer. Below this level, 0.2% urea, the glycol yield was reduced and sugar utilization was decreased when the calcium carbonate was omitted. There was no difference in the final pH when the calcium carbonate was added or omitted. This was true for both 0.2 and 0.3% of urea.

TABLE 6 - Effect of omitting calcium carbonate when urea is used as the nitrogen source in a 15% malt hydrolyzed wheat mash.

UREA grams per 100 cc medium	$CaCO_3$ grams per 100 cc medium	Final pH	Final Sugar x	% Glycol	Glycol Mean
.2	.5	4.95	≠ ≠	3.03	
"	.5	5.1	≠ ≠	3.04	
"	.5	5.1	≠ ≠	3.09	3.05
.2	None	5.0	≠ ≠ ≠	2.82	
"	"	5.0	≠ ≠ ≠	2.71	
"	"	5.1	≠ ≠ ≠	2.67	2.73
.3	.5	5.2	≠	3.37	
"	.5	5.3	≠	3.34	
"	.5	5.2	≠	3.16	3.29
.3	None	5.2	≠	3.35	
"	"	5.3	≠	3.20	
"	"	5.2	≠	3.30	3.28

x This column represents the relative amounts of sugar remaining in the fermentation liquors.

5. A Study of Weathered Wheat, Barley, Potatoes and Beet Molasses as Sources of Raw Material for the Fermentation.

(a) Weathered Wheat.

A study was made by the Plant Science department, using Aerobacillus polymyxa, to determine the relative value of weathered and unweathered wheat in the fermentation. Although they could attribute no diferences in glycol yield to degree of weathering it was noted that strain #500 gave better results with the fall threshed samples. It was thought advisable to test the effect of weathered wheat on the Aerobacter aerogenes fermentation.

Due to the late harvest and early winter of 1942 it was necessary to leave much of the wheat unthreshed in the field until spring. It was thus subjected to severe weathering, including frost damage, and in some instances to germination. Fall and spring threshed samples from the same field were chosen, representing different localities in the province and different degrees of weathering. The results are reported in Table 7. Although the yields were not consistent the fall threshed samples gave better results for the majority of samples tested. The over all average was only slightly higher for the unweathered samples and indicates the suitability

TABLE 7 - Glycol yield in grams from 15 grams weathered and unweathered wheat.

Sample	Grams Glycol Weathered	Unweathered
1	3.71	3.69
2	3.11	3.51
3	3.68	3.72
4	3.60	3.70
5	3.96	3.88
6	3.68	3.83
Average	3.62	3.72

of low grade [illegible] wheat for this fermentation.

(b) Barley

An experiment was carried out to determine the comparative value of barley and wheat as a carbohydrate source in Aerobacter aerogenes fermentation. For this test two varieties of barley, Trebi and Newal grown at Edmonton, and one variety of wheat, Garnet, were mashed, malt hydrolyzed, and fermented. Garnet wheat was chosen because it was

TABLE 8 - Comparative yields of glycol from wheat and barley mashes.

		Grams Glycol from 10 grams grain	
		10% Mash	15% Mash
Garnet Wheat		2.55	2.53
		2.69	2.56
		2.65	2.50
	Mean	2.63	2.53
Newal Barley		2.50	2.33
		2.48	2.28
		2.45	2.38
	Mean	2.48	2.33
Trebi Barley		2.57	2.33
		2.50	2.41
		2.46	2.31
	Mean	2.49	2.35

found to give slightly better results than the other wheat varieties. Tests were made on 10% and 15% mashes of both barley and wheat. The results appear in Table 8.

The average yield was 2.58 grams glycol from 10 grams wheat mash, 2.41 grams glycol from 10 grams barley mash. Thus the average yield of glycol from barley was 6.6% lower than from wheat. This may be explained by the fact that barley contains 6 to 8% less starch than wheat. However,

Table 5 - Comparative values of elapsed time between
and Delay session.

Normal speech			
	Mean		
Tonal Delay			
	Mean		
Tonal Delay			
	Mean		

found to give stability after reaching that value
about variables. Tests were made on up to 100
number of such b-duty and test. Results are
ance in Table 5.

The response time was also found
from 32 to the mean value of 0.49 when
stance Delay used. Once the learning
ral Time Delay was 4.0 time more
This was explained by the fact that
tains 8 to 20 time steps but not 4. Therefore,

since barley generally yields more starch per acre than does wheat, it would appear that barley could be used more economically in the fermentation than wheat.

Similar results were obtained using the organism Aerobacillus polymyxa (22). There was no real difference in yield between the two varieties of barley.

(c) Potatoes

A brief study was made concerning the utilization of potatoes as a carbohydrate source for Aerobacter fermentation. Although the investigation was very incomplete the results would indicate that this starchy material is not wholly satisfactory for this fermentation. Complete saccharification of the cooked potato mash was very difficult to obtain. This is an important factor since the organism Aerobacter aerogenes will not ferment starch or dextrin . Several saccharifying agents including sulphuric acid, barley malt, and diastase were used. Complete conversion of the starch to sugar was not given by any one of these alone.

The potatoes were sliced and cooked in the autoclave at 15 lbs. pressure for 30 minutes,

following which they were thoroughly mashed and the saccharifying agent added. Distilled water was added to make a 50% mash by weight. Acid hydrolysis gave only partial conversion -- not exceeding 30% of theoretical. Malt hydrolysis (itself incomplete) followed by acid hydrolysis gave complete conversion of the starch to sugar, which was indicated both by the iodine test and sugar analysis. Hydrolysis of the potato mash with diastase produced a mash which gave a negative starch test with iodine but positive for dextrins. Acid hydrolysis of this mash increased the reducing sugar content from 5.5 to 7.3%. Glycol yields varying from 2.42 to 2.66 grams per 50 grams of potatoes (approximately 11 grams dry matter) were recovered by analysis after fertmentation.

(d) Beet Molasses.

Some fermentation tests were carried out using beet molasses as the sugar source. The beet molasses tested was secured from the Canadian Sugar Factories at Raymond, Alberta. The fermentation medium was prepared by diluting one part of molasses with four parts of distilled water. Salts and urea were added at the time of inoculation. These fer-

mentations required an incubation period of about 72 hours. An anti foam agent was required to prevent foaming during aeration.

These tests indicate that beet molasses can be utilized quite efficiently in Aerobacter fermentations. Yields ranging from 19.4 to 20.4 grams of glycol together with 5.3 to 5.6 grams of alcohol were recovered from 100 grams of molasses containing approximately 55 grams of sugar. Acid hydrolysis of the beet molasses medium prior to fermentation did not improve the glycol yields. Several fermentations were made on unsterilized beet molasses medium and yields comparable with those from sterilized medium were obtained.

Beet molasses should prove to be a good source of raw material for glycol production by means of Aerobacter fermentation, and may provide the basis for a small glycol production plant in Alberta. The approximate total yearly supply of beet molasses in Alberta is about 10,000 tons. At present price levels (Table 9) it is a cheaper source of raw material than wheat or barley (at least to Canadian consumers). At the price charged for export to the United States it is more costly than barley.

Molasses also has several other factors in its favor. It can be fermented directly without first being hydrolyzed, which in the case of wheat and barley involves several steps, including grinding, cooking, saccharification, etc. Acid hydrolyzed mashes must be adjusted to a proper pH before they can be fermented. If malt hydrolysis of starch is used resterilization is essential since the organism Aerobacter aerogenes can not compete with the contamination introduced with the malt. These steps all add to the cost of fermentation when grain or other starchy materials are used. It is probable that beet molasses need not be sterilized prior to fermentation since several fermentations were made successfully without sterilization. These facts indicate that beet molasses is a good potential source of raw material for glycol production by means of Aerobacter fermentation.

TABLE 9 - The relative cost of beet molasses, wheat and barley per ton of fermentable material (sugar or starch) based on quoted prices.

	Price per ton	Cost per ton sugar
Beet Molasses		
To beet growers	$13.50	$24.55
To feed manufacturers	16.50	30.00
To yeast manufacturers	19.85	36.00
For export to U.S.	28.50	52.00
	Price per bus.	**Cost per ton starch**
Wheat		
#1 northern	$ 1.25	$75.00
Feed	1.00	60.00
Barley	$ .50	$43.40

SUMMARY

Aeration is necessary for a fast and efficient fermentation.

The role of aeration is probably not only a physical one as there are indications that it has some chemical effect on the fermentation.

The organism Aerobacter aerogenes can tolerate an air mixture containing as high as 25% carbon dioxide without seriously inhibiting the fermentation. This indicated that the volume of sterile air required for aeration may be reduced by recycling the air through the fermentation medium.

Foaming during aeration is caused by the proteins present in the hydrolyzed mash. It can be controlled either by enzyme hydrolysis of the proteins or by the addition of a suitable anti-foam agent (lubricating oil) without impairing glycol yields.

The use of oleic acid to prevent foaming gives a slightly lower yield of glycol, but probably not significantly lower than when no anti-foam agent is used. Oleic acid is more costly than lubricating oil.

Barley malt prepared in the laboratory and dried at a low temperature to prevent destruction of the proteolytic enzymes does not effect sufficient breakdown of the proteins to

effectively control foaming when used to saccharify wheat mashes.

The organism Aerobacter aerogenes does not survive for an appreciable time when stored in "lyophile" form.

In dextrose medium the rate of fermentation increased as the amount of urea added to the medium increased, up to the limit added.

In malt hydrolyzed wheat mash 0.3% urea gave an optimum rate of fermentation.

Calcium carbonate increased the rate of fermentation when 0.2% urea was added to malt hydrolyzed wheat mashes but had no beneficial effect when 0.3% urea was added.

Weathered and poor quality wheat gave approximately as high yields of glycol as good quality wheat in Aerobacter fermentations.

Barley may be used more economically than wheat in the fermentation because of its higher average yield of starch.

Potatoes are not considered a good source of raw material for Aerobacter fermentations since it is difficult to obtain complete saccharification of the potato starch.

Beet molasses can probably be used advantageously in this fermentation. Results indicate that:

1. Sterilization of molasses medium before fermentation is not neceasary.
2. It is readily fermented without pre-hydrolysis.
3. Acid hydrolysis of the molasses does not increase yields.
4. It is a relatively cheap source of raw material.

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LITERATURE CITED

1. ANDERSON, C.G. Introduction to Bacteriological Chemistry. E.S. Livingstone, Edinburgh. 1939.

2. BERGY'S Mannual of Determinative Bacteriology. 5th Edition. The Williams and Wilkins Co., Baltimore. 1939.

3. BLACKWOOD, A.C. The production of 2,3-butylene glycol by Aerobacter fermentation. M.Sc. thesis, University of Alberta. 1944.

4. CALLAHAM, J.R. Butylene Glycol - postwar chemical or new source of butadiene? Chemical and Metallurigical Engineering. November, 1944.

5. CORNS, W.G. Estimation of 2,3 butylene glycol and acetylmethyl carbinol and certain other substances in the fermentation of grain with some comparative results. M. Sc. thesis, University of Alberta. 1944.

6. ELSER, W.J., THOMAS, R.A., and STEFFEN, G.I. The dessication of sera and other biological products in the frozen state, with the preservation of the original qualities of products so treated. Jour. Immunol. 28: 433-473, 1935.

7. FLOSDORF, E.W. and MUDD, S. An improved procedure and apparatus for preservation of sera, microorganisms and other substances. Jour. Immunol. 34: 469-489. 1938.

8. FULMER, C. and KENDALL, A.R. Production of 2,3-butylene glycol by fermentation. Ind. and Eng. Chem. 25: 798-800. 1933.

9. HARDIN, A. and WALPOLE, G.S. Chemical action of Bacillus lactis and aerogenes (Escherich) on glucose and mannitol. Proc. Roy. Soc. (London) B. 77: 399-405. 1905.

10. HEYDEN CHEMICAL CORPORATION. Report on 2,3-butylene glycol fermentation. Garfield, New York. January 1. 1943.

11. JOSEPH E. SEAGRAMS AND SONS, Inc. Summary of 2,3-butylene glycol research for the period of February 1942 to January 1943. Louisville, Kentucky. January, 1943.

12. KENDALL, A.R. The biochemistry of the production of 2,3-butylene glycol by fermentation. Chem. Abst. 29: 5217. 1933.

13. KLUYVER, A.J. The Chemical Activities of Microorganisms. University of London Press, 10 Warwick Lane, London. 1931.

14. LEDINGHAM, G.A. Culture Drying, National Research Council Report No. 11 - 8 - 43. 1943.

15. NATIONAL RESEARCH COUNCIL et al. Canadian investigations on the production of 2,3-butylene glycol. December, 1942.

16. NORTHERN REGIONAL RESEARCH LABORATORY. Production of 2,3-butylene glycol from January 1941 to April 1942. U.S.D.A. Peoria, Ill. 1942.

17. REYNOLDS, H. and WERKMAN, C.H. The intermediate dissimilation of glucose by Aerobacter indologenes. Jour. of Bact. 33: 603-614. 1937.

18. SCHENELY RESEARCH INSTITUTE INC. Research on the production of butadiene from 2,3-butylene glycol from grain. Lawrenceburg, Ind. December 1942.

19. SCHENELY RESEARCH INSTITUTE INC. Research on the production of butadiene from 2,3-butylene glycol from grain. April, 1943.

20. STANIER, R.Y. and FRATKIN, SYBIL B. Studies on the bacterial oxidation of 2,3-butandiol and related compounds. Can. Jour. Research, B. 22: 140-153. September, 1944.

21. UNIVERSITY OF ALBERTA. Studies on the production of 2,3-butylene glycol from grain. Report No. 3. Edmonton, Alberta. September, 1943.

22. UNIVERSITY OF WISCONSIN. 2,3-butylene glycol. Wisconsin report No. 1. Madison, Wis. January 1943.

23. VERHAVE, T.H. 2,3-butylene glycol prepared using Clostridium polymyxa or Aerobacter aerogenes. Chem. Abst. 24: 1652. 1928.

24. WAKSMAN, S.A. and DAVIDSON, W.C. Enzymes. Bailliere, Tindall and Cox, 8 Henrietta St., Covent Garden, W.C.2, London. 1926.